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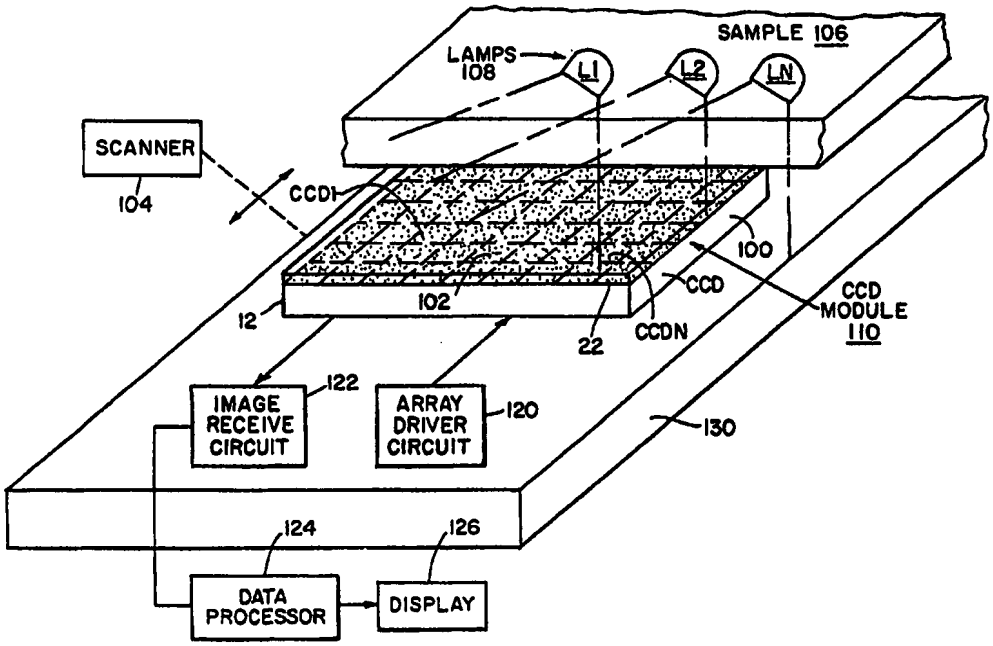

(57) Abstract

A method and apparatus for ultrasensitive detection and quantification of particle emissions from an emitter to produce a large format high resolution digital image of the spatial distribution of detected emissions is described. In one embodiment, the detector apparatus consist of a plurality of solid state imaging devices, such as an array of charge-coupled devices arranged in close proximity to the emitter in a manner to produce the relatively large format digital image without the need for a lens between the emitter and detector.

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METHODS AND APPARATUS FOR DETECTING AND IMAGING PARTICLES

Background of the Invention

The present invention is directly applicable to both healthcare and scientific fields, such as, molecular imaging. Regarding healthcare, previous attempts at replacing conventional x-ray photography with digital imaging systems such as computer tomography (CT) and magnetic resonance imaging (MRI) have been somewhat limited because of the extremely high detection sensitivity required. Even with the advent of CT and MRI digital imaging techniques, it has been estimated that over 90% of the image diagnostic cases in healthcare continue to be provided by conventional x-ray photography. A need exists therefore for enhanced sensitivity in medical radiography since the patient must be imaged in a very short time in order to prevent blurring caused by the motion of internal organs (typically less than 1/100 sec.), as well as reducing the risks associated with radiation exposure.

An increase in sensitivity also offers many advantages which can be exploited in scientific fields such as x-ray crystallography, electron microscopy and the imaging of labeled molecules. With increased sensitivity complicated molecular structures can be analyzed by x-ray diffraction imaging much faster than conventional scintillation counting. Moreover, with increased sensitivity, the radiation source intensity required is significantly lower which allows transmission electron microscopy without damaging the sample with electron rays. That is, organic crystals and biopolymers are not broken down during analysis with an electron microscope, thereby allowing the use of the full theoretical resolution of electron microscopes.

Regarding molecular imaging, it is often desirable to rapidly detect and quantify one or more molecular structures in a sample. The molecular structures typically comprise ligands, such as cells, antibodies and anti-antibodies. Ligands are molecules which are recognized by a particular receptor. Ligands may include, without limitation, agonists and antagonists for cell membrane receptors, toxins, venoms, oligo-saccharides, proteins, bacteria, and monoclonal antibodies. For example, a DNA or RNA sequence analysis is very useful in genetic and disease diagnosis, toxicology testing, genetic research, agriculture and pharmaceutical development. Likewise, cell and antibody detection is important in disease diagnosis.

A. Gel Electrophoresis

Analysis of many such molecular structures is often accomplished via gel electrophoresis; whereby biomolecules such as proteins and nucleic acids are separated by 1) disposing a sample, having an inherent charge polarity, into a suitable gel, 2) applying an electrical potential across an electrode pair bounding the gel and 3) allowing the charged molecular components of the sample to migrate through the gel under the influence of the applied electric field. Separation is achieved simply from the differential migration velocities exhibited by the molecular components. Once separated, the molecular components within the sample can be identified by detecting and quantifying the distribution of charged particles emitted from the labeled molecular components.

B. Autoradiography

Several imaging procedures have been developed for detecting and quantifying the spatial particle emission distributions of such molecular samples. The most

conventional imaging technique is autoradiography, whereby the molecular constituents are labeled with radioisotope tags such as ^{32}P and ^{35}S, and subsequently exposed to photographic film. Autoradiography provides a moderate resolution image over a relatively large format area, but is plagued by several disadvantages. Due to the relative insensitivity of photographic film to beta particles, the time required for exposure can vary from hours to days. The exposure can be accelerated with the use of intensifying screens, yet spatial resolution is significantly degraded. Typical spatial resolution for autoradiography is on the order of 1 to 10 millimeters. In addition, the resulting photographic image has to be read by a digital scanner for automation purposes.

C. Gas Phase Ionization Beta Detection

Gas phase ionization beta detection is another procedure for imaging radiolabeled molecules as disclosed in the following patent;

[1] Bolon, *Process and Apparatus for Measuring Surface Distributions of Charged Particle Emitting Radionuclides,* United States Patent Number: 4,670,656, June 2, 1987, (incorporated by reference herein).

In this technique, beta particles are detected in two separate phases, and the source of beta emission is then calculated by back propagation from the detector plates to the beta source. Since the beta particles are emitted at all angles from the surface horizontal, the back propagation results in a blurring effect that limits the resolution, and is a function of the distance between the detector and the sample. Here the spatial resolution is on the order of 0.1 to 1 millimeters (a factor of 10 better than film) and provides a quantitative digital

image. The sensitivity is approximately 10 to 100 times better than film, yielding quantitative digital images at least 10 times faster than film (minutes to hours).

D. Storage Phosphor Screen Technology

Another method of quantitative detection and imaging of radiolabeled molecules is the storage phosphor screen technology, or phosphorimagers. Here a screen of phosphor is placed in a metastable state when exposed to a radioactively labeled sample. Following exposure, the screen is read by exciting the phosphor by a laser and imaging the photons emitted as the electrons return to the ground state. Both strong ^{32}P and weak (^{14}C and ^{35}S) beta particles can be imaged. The spatial resolution and sensitivity is comparable to the gas phase beta detectors, and offers many advantages. First, the variable limiting the number of isotopic scans is the number of imager plates. In essence many samples can be placed on a number of imaging plates. Since the readout process is very short (approximately 5 minutes), compared with the time required for exposure of the storage phosphor matrix to beta particle emission (tens of minutes to hours), the machine is utilized more efficiently. Second, quantitation of beta particles of lower energy are more amenable to phosphorimagers. Tritium (^{3}H) can be imaged on a phosphorimager, however, in this case the imaging plate is not reusable. Thus, it is very expensive to detect ^{3}H on a phosphorimager. Another disadvantage is optical self attenuation, being a function of the screen thickness.

E. Fluorescent Detection Systems

To circumvent the disadvantages of the above detection and imaging methods based on radiolabeling, several fluorescent detection systems have been developed.

Here the molecular components are labeled with fluorescent dyes with distinct emission bands. For example, in DNA sequencing, 4 fluorophores are used to label each of 4 dideoxy sequencing reactions. Following gel electrophoresis, the sample is radiated with a laser and images are produced from signals obtained with a photomultiplier tube (PMT). The wavelength selectivity, corresponding to distinct molecular constituents, is obtained by positioning bandpass filters in front of the PMT. Further automation can be achieved by multiplexing several samples per run, as well as automating the base calling with computer algorithms, characteristic of the Applied Biosystems, Inc. DNA sequencer.

More recently, several fluorescent detection and imaging techniques have been disclosed utilizing charge-coupled-device (CCD) cameras in conjunction with gel electrophoresis as described in the following listed patents (incorporated by reference herein):

[2] Mackay, *Analysis of Samples by Electrophoresis Using a Charge Coupled Device,* United States Patent Number: 4,874,492, October 17, 1989.

[3] Yamamoto, *et al., Electrophoresis Pattern Analyzer for Genetic Material,* United States Patent Number: 5,061,067, October 29, 1991.

[4] Pentoney, Jr. *et al., Detection of Radioisotope Labeled Components in a Capillary,* United States Patent Number: 5,143,850, September 1, 1992.

In each case a cooled CCD camera is used to image the fluorescently tagged molecular constituents in the gel. Advantages of a CCD-based detection and quantitative imaging approach include: 1) avoidance of mutagenic radioactive labels, 2) high sensitivity (comparable to PMT), 3) fast exposure time (seconds), 4) linear response over wide dynamic range (5 orders of magnitude), 5) low

noise, 6) high quantum efficiency, and 7) fast data acquisition (megapixels/sec).

Several articles have recently appeared describing the employment of CCD cameras for electrophoresis applications including the following,(incorporated by reference herein):

[5] Jackson, P., Urwin, V.E., Mackey, C.D., Rapid Imaging Using a Cooled Charge-Coupled Device of Fluorescent Two-dimensional Polyacrylamide Gels Produced by Labeling Proteins in the First Dimensional Isoelectric Focusing Gels with the Flurophore 2-Methoxy-2, 4-Diphenyl-3(2H) Furanone, *Electrophoresis* 9(7), 330-339, 1988.

[6] Chan, K.C., Koutny, L.B., and Yeung, E.S., On-Line Detection of DNA in Gel Electrophoresis by Ultraviolet Absorption Utilizing a Charge-Coupled Device Imaging System, *Anal. Chem.*, 63, 746-750, 1991.

where the gel is radiated with a UV laser and the absorption of the DNA constituents is recorded digitally with a CCD camera providing on-line detection and imaging,

[7] McGregor, D.A. and Yeung, E.S., Interactive Control of Pulsed Field Gel Electrophoresis via Real Time Monitoring, *Anal. Chem.*, 64, 1-6, 1992.

where a CCD camera has been used for pulsed field gel electrophoresis wherein the electric field is alternated interactively causing the DNA fragments to snake through the gel at approximately 10 times faster than conventional electrophoresis,

[8] Cheng, Y.F., Piccard, R.D. and Vo-Dinh, T., *Appl. Spectrose* 44:755-765, 1990.

[9] Sweedler, J.V., Shear, J.B., Fishman, H.A., Zare, R.N. and Scheller, R.H., Fluorescence Detection in Capillary Zone Electrophoresis

Using a Charge-Coupled Device With Time-delayed Integration, *Anal. Chem.*, 63, 496-502, 1991.

[10] Kostichka, A.J., Marchbanks, M.L., Brumley, Jr., R.L., Drossman, H. and Smith, L.M., High Speed Automated DNA Sequencing in Ultrathin Slab Gels, *Biotechnology,* 10:78-81, 1992.

[11] Karger, A.E., Harris, J.M. and Gesteland, R.F., Multiwavelength Fluorescence Detection for DNA Sequencing Using Capillary Electrophoresis, *Nuc. Acids Res.*, 19:4955-4962, 1991.

where the presence of a CCD camera has appeared in capillary gel electrophoresis applications wherein very thin fused silicon capillaries are filled with denatured polyacrylamide gel which facilitate substantially larger electric fields due to the efficient heat transfer properties of the small capillaries. Consequently, throughput is increased by an order of magnitude due to the combination of faster molecular mobilities in the gel (26 times faster migration), multiple wavelength fluorophores, and on-line parallel CCD camera imaging. Also, no moving parts are required, such as a filter wheel, since a wedge prism assembly is used to provide distinct emission bands for CCD camera imaging. Alternatively, the multiple wavelength emissions can be discriminated by calculating the condition number to avoid the use of bandpass filters.

Collectively, these CCD camera-based approaches for detection and quantitative imaging of particle emissions from molecular samples circumvent many of the limitations of autoradiography, gas phase ionization beta detection, phosphorimaging and PMT fluorescence detection. However, limitations do persist including the low collection efficiency inherent to any CCD technique having long focal lengths (approximately 75 cm) from the sample (emitter) to the detector, cryogenics required to suppress CCD dark currents, and the expense incurred for the lens optics required with long focal lengths.

F. CCD Lens-Based Document Imaging

CCD technology utilizing optical lenses for imaging documents has been well documented and applied in conventional document scanners, copying machines, and facsimile machines, as disclosed in the following patents (incorporated herein by reference):

[12] Suzuki, *Image Reading Device,* United States Patent Number: 4,772,958, September 20, 1998.

[13] Suzuki, *Image Reading Apparatus,* United States Patent Number: 4,675,745, June 23, 1987

[14] Hirota, *Image Reading Apparatus Having Plural Line Image Sensor Chips,* United States Patent Number: 5,003,380, March 26, 1991.

[15] Hornbaker, III *et al.*, *Scanning Apparatus Using Multiple CCD Arrays and Related Method,* United States Patent Number: 5,144,448, September 1, 1992.

[16] Suzuki *et al.*, *Image Reader Using a Plurality of CCDs,* United States Patent Number: 4,849,820, July 18, 1989.

[17] Suzuki *et al.*, *Image Reader Using a Plurality of CCDs,* United States Patent Number: 4,774,592, September 27, 1988.

Although these lens-based CCD approaches often employ large format imaging sensors, they are not applicable to imaging charged particles emitted from radioisotope, chemiluminescent or fluorescent labeled molecular samples.

Summary of the Invention

The present invention provides an ultrasensitive detection, quantitative imaging and spectroscopy method and apparatus that yields high resolution digital images of the spatial distribution of particle emissions from relatively large format samples in a minimal amount of time and expense.

The apparatus is comprised of a lens-less imaging array comprising a plurality of solid state imaging devices, such as an array of CCDs, charge injection devices (CIDs), photodiode arrays, amorphous silicon sensors or the like. The array is disposed in close proximity to the sample and is comparable in size to the area of the sample to be imaged. In this manner a relatively large format digital image of the spatial distribution of sample emission or absorption is produced without requiring the use of one or more lenses between the sample and the imaging array. The apparatus offers 1) high sensitivity (10^3 to 10^4 times more than photographic film, 10 - 100 times more than gas phase ionization detection and phosphorimagers), 2) high throughput (seconds instead of minutes to hours for gas phase ionization detectors and phosphorimagers, and hours to days for photographic film), 3) linear response over a wide dynamic range (4 to 5 orders of magnitude), 4) low noise, 5) high quantum efficiency and 6) fast data acquisition. Moreover by placing the imaging array in proximity to the sample, the collection efficiency is improved by a factor of at least 10 over any lens-based

technique such as found in conventional CCD cameras. That is, the sample (emitter or absorber) is in near contact with the detector (imaging array), eliminating conventional imaging optics such as lenses and mirrors. The inventive apparatus can be used for detecting and quantitatively imaging radioisotope, fluorescent, and chemiluminescent labeled molecules, since a lens-less CCD array apparatus is highly sensitive to both photons and x-ray particles. Hence a single imaging instrument can be used in conjunction with numerous molecular labeling techniques, ranging from radioisotopes to fluorescent dyes.

Two categories of embodiments of the invention are disclosed. The first embodiment entails a *static* platform whereby a plurality of imaging devices are arranged in a relatively large format area comparable to the sample size.

The second category of embodiments of the invention entails a *dynamic* platform that enables a smaller set of imaging devices to image a relatively large format sample by moving either the array of imaging devices or sample, relative to one another.

In summary, the dynamic embodiment of the invention generally concerns a method and apparatus for ultrasensitive detection, high resolution quantitative digital imaging and spectroscopy of the spatial and/or temporal distribution of particle emissions or absorption from/by a sample in a relatively large format. The apparatus of the invention includes:

a) a large area detector array for producing a relatively large image of detected particle distribution without the use of optical lenses;

b) a scanner for moving either the sensor array or the sample in a manner for efficient imaging; and

c) a source of energy for exciting the sample or providing absorption by the sample.

Optimally the ratio of detector array size to sample image is 1 for a static format and less than 1 for a dynamic format.

Brief Description of the Drawings

FIG. 1A is a schematic sectional view of a portion of a static embodiment of the invention.

FIG. 1B is a plan view of a static embodiment, showing 4 columns of CCD chips.

FIG. 1C is a plan view of a static embodiment with 4 columns and 5 rows of CCD chips.

FIG. 2A is a schematic diagram illustrating the geometry of a lens coupled detector system of the prior art.

FIG. 2B is a schematic diagram illustrating the geometry of a lens-less detector system of the present invention.

FIG. 3 is a schematic diagram of a dynamic embodiment of the invention.

FIG. 4 is a schematic of an alternate embodiment of the invention.

FIG. 5 is a sectional view of a conventional wire bond technique applied to the present device.

FIG. 6 is a sectional view of a modification of FIG. 5 to accommodate wire bonding of the present detector system.

FIG. 7 is a sectional view of a low profile wire bond of the present invention.

FIG. 8 is a sectional view of a detector/sample using the bonding system of FIG. 7.

FIG. 9 is a sectional view of a detector/sample with a low profile bond and isolated imaging on a dynamic platform.

FIG. 10 is a sectional view of a detector/sample for a backside illuminated approach.

Description of the Invention

Sensor System

A preferred embodiment of a sensor system 10 of the invention consists of a plurality of CCD arrays CCD1...CCDN assembled in a large format module as illustrated in FIGS. 1A-1C. Individual CCD arrays are closely aligned and interconnected in particular geometries to form a relatively large (greater than $1cm^2$) format imaging sensors of the linear array type 10B or the two dimensional row and column type 10C shown in FIGS. 1B and 1C respectively.

Each CCD array CCD1...CCDN is formed, in the conventional manner, by growing and patterning various oxide layers 14 on a Si wafer/substrate 12. CCD gate electrodes 10 are then formed by deposition of polysilicon or other transparent gate material on the gate insulator or field oxide 14. A dielectric or polymer layer 18, preferably of light transmissive material such as silicon nitride or glass, SiO_2 or polyamide is then formed over the electrodes 16.

Preferably, in a labeled molecule embodiment a filter shown in dotted lines 17, which may be formed of an aluminum or tungsten metal grating, or dielectric multilayer interference filter, or absorption filter, is formed in the dielectric layer 18 between the surface and the metal electrode 16. The filter is adapted to block the excitation radiation and pass the secondary emission from the sample 20. In a static platform embodiment, the

sensor module remains fixed with respect to the sample. Hence to achieve the relatively large imaging format, a plurality of imaging devices CCD1...CCDN must be arranged in a module as illustrated in FIGS. 1B and 1C. The module can be packaged for easy installation to facilitate multiple modules, each for specific applications. Various tiling strategies have been documented and can be employed to minimize the discontinuity between devices, such as described in:

[18] Burke, *et al.*, "An Abuttable CCD Imager for Visible and X-Ray Focal Plane Arrays," IEEE Trans. on Electron Devices, *38*(5):1069 (May 1991)

As illustrated in FIG. 1A, a sample 20 is placed in proximity to the CCD array sensor 10. For example, the sample may be the chest of a patient for clinical radiology applications, or a gel having labeled molecular constituents for analysis. The sample can be excited by an external energy source or can be internally labeled with radioisotopes emitting energetic particles or radiation, or photons may be emitted by the sample when labeled with fluorescent and chemiluminescent substances. Conversely, direct absorption may be used to determine their presence. In this case, the absence of illuminating radiation on the detector may constitute the presence of a particular molecule structure. Preferably the sample can be physically separated from the CCD detector by (an optional) thin isolation plate 22, such as glass or quartz, which is transparent to the particle emission.

The CCD detection and imaging arrays CCD1-CCDN generate electron-hole pairs in the silicon 12 when the charged particles or radiation of energy $h\upsilon$ shown by the "asterisk" 32 arising from or transmitted by the sample are incident (arrows 30) on the CCD gates 16. Alternatively, the CCDs can be constructed in a back

illuminator format whereby the charged particles are incident in the bulk silicon 12 for increased sensitivity. The liberated photoelectrons 34 are then collected beneath adjacent CCD gates 16 and sequentially read out on a display (not shown) by display module 36 in the well-known manner.

Silicon based CCD's are preferred as the solid state detection and imaging sensor primarily due to the high sensitivity of the devices over a wide wavelength range of from 1 to 10,000Å wavelengths. That is, silicon is very responsive to electromagnetic radiation from the visible spectrum to soft x-rays. Specifically for silicon, only 1.1 eV of energy is required to generate an electron-hole pair in the 3000 to 11000Å wavelength range. Thus for visible light, a single photon incident on the CCD gate 16 will result in a single electron charge packet beneath the gate, whereas for soft x-rays, a single beta particle (typically KeV to MeV range) will generate thousands to tens of thousands of electrons. Hence the silicon CCD device provides ultrasensitive detection and imaging for low energy alpha or beta emitting isotopes (^{3}H, ^{14}C, ^{35}S) as well as high energy alpha or beta emitting isotopes (^{32}P, ^{125}I). Consequently, the CCD is both a visible imager (applicable to fluorescent and chemiluminescent labeled molecular samples) and a particle spectrometer (applicable to radioisotope labeled samples as well as external x-ray radiated samples). In fact the CCD is the only sensor that can provide simultaneous imaging and spectroscopy in the same image.

In addition to the high sensitivity, the CCDs offer a wide dynamic range (5 orders of magnitude) since the charge packet collected beneath each pixel or gate 16 can range from a few to a million electrons. Furthermore, the detection response is linear over the wide dynamic range

which facilitates the spectroscopy function, since the amount of charge collected is directly proportional to the incident photon energy. Hence, no reciprocity breakdown occurs in CCDs, a well-known limitation in photographic film.

SNR Calculations

Several signal-to-noise ratio (SNR) expressions are presented below to convey the advantages of the preferred lens-less CCD embodiment for ultrasensitive detection and imaging of samples in a relatively large format.

·X-ray Excitation

The first approach requires either external x-ray sample excitation or the attachment of a radioactive label to the sample constituents. Here the pixels (gates) 16 on the CCD arrays accumulate electrical charge proportional to the radiation products absorbed from the sample. Hence, charge particles are detected almost instantaneously by automatically addressing and reading the charge.

The SNR for the lens-less CCD approach used with x-ray excitation can be expressed once the integration time is determined. Assume the sample is labeled with ^{32}P. Let N_l = sample labeling density (10^{11} labeled molecules/cm^2); T = ^{32}P decay time (18 days); Ap = pixel area (27 μm)^2; A_l = area of active labeling per molecular component; A = total area of single molecular component site (including guard pixels); t = integration time (sec); I_d = dark current (1nA/cm^2); q = electron charge (1.6×10^{-19} coul) and σ_r^2 = read noise (10 electrons/pixel). The number of radioactive events within integration time t occurring in an active area A_l is given by

$$N_e(t) = \frac{1}{2} N_t A_1 (1 - e^{-t/T})$$

and for $t \ll T$

$$N_e(t) \approx \frac{1}{2} N_t A_1 \frac{t}{T}.$$

To achieve high probability of detection at least 10 events are required, yielding the net integration time for an approximate $(100\mu m)^2$ label area $A_1 = 16A_p$

$$t = \frac{2(10)T}{A_1 N_t} \approx 3 \text{sec}.$$

Caution must be exercised in designing the array to avoid crosstalk between pixels, since the beta particle creates a trail of photocharge that is collected across several pixels. The corresponding SNR calculated with the guard pixels to avoid crosstalk ($A = 20^2 A_p$) can be expressed

$$SNR = \frac{SNe(t)}{\sqrt{SNe(t) + \sigma^2_r + \frac{I_d t A}{q}}} \approx 122$$

where S = electrons generated per beta particle (single event). From experimental results, S = 90,000 electrons for a 1.7 MeV beta particle.

The dark current contribution to the noise can be decreased and crosstalk suppressed by framing the CCD very quickly using a shorter integration time $\tau \ll t$. By fast framing, the CCD pixels become single event detectors

since the number of collected photoelectrons in a pixel remains larger than either the dark current or read noise and the probability of one or more events is small in the duration of τ seconds. Although the read noise increases, the charge-to-voltage noise associated with a single pixel readout can be less than 10 electrons. Also, the pixels can be designed to minimize the variation in electrons collected per beta particle event.

Such technique is well suited to the x-ray excitation mode since the beta particle events are relatively infrequent and easily distinguished from noise frames. Moreover, crosstalk is further suppressed by the technique since the probability of beta events occurring simultaneously in neighboring sample component sites decreases with decreasing integrating time. The use of lower energy beta particles (^{35}S) will also suppress crosstalk. Therefore the density of the labeling can be increased to accommodate thousands of distinct molecular components on a single sample when employing single event detection.

Consequently, a ^{32}P labeled sample can be detected in seconds time throughout thousands of sites on a large format CCD imaging module operating at room temperature with sufficient SNR. A similar analysis can be conducted for a sample externally excited by a x-ray source by substituting the radiated energy level incident on the imaging module for the signal electron density S.

·Fluorescent Excitation

The second approach analyzed involves the use of fluorescent-labeled receptors attached to the sample components. The fluorescent labels can be attached covalently or through intercalation.

The detection procedure for using the fluorescent labels begins by analyzing the optical absorption spectrum

of the chosen label. The regions in the spectra of high absorption are used to select the illumination source wavelength. For many of the labels, an ultraviolet (UV) light source provides maximum absorption and emission is in the red spectrum (e.g., 630 nm). These spectral regions of excitation and detection enhance SNR, since the polysilicon gates of the CCD substrate naturally absorb UV light and the line grating fabricated on the chip can provide optical filtering near the 630 nm region. Consequently, sensitivity can be improved by employing fluorescent markers, choosing the optimal excitation wavelength and the corresponding detection filter implemented directly on the CCD chip. Finally, the charge accumulated at the pixels is proportional to the number of photons detected from the fluorescence of the molecular constituents of the sample.

The SNR for the lens-less CCD approach in the fluorescent mode of operation is given by

$$SNR=\frac{P\sigma N_t\eta_d\eta_f\eta A_p t}{\sqrt{P\sigma N_t\eta_d\eta_f\eta A_p t+\frac{I_d A_p t}{q}+\sigma^2_r}}$$

where P = pump power (laser or bulb) (photons/cm^2·sec); σ = absorption cross section of the dye (20×10^{-16} cm^2); η_d = detection quantum efficiency (4 electrons/10 photons at 700 nm emission); η_f = fluorescence quantum yield (1); η = CCD collection efficiency (50%); I_d = dark current noise (1nA/cm^2 at room temperature; h = Planck's constant (6.6×10^{-27} erg·sec).

Thus, in order to obtain a SNR = 100 to ensure high detection probability, the power density required of the illumination source for a one second integration time is given by:

$$P=\frac{1}{2}\left[\frac{SNR^2}{\alpha A_p t}+\left[\left(\frac{SNR^2}{\alpha A_p t}\right)^2+\frac{4I_d SNR^2}{q\alpha^2 A_p t}\right]^{\frac{1}{2}}\right]$$

$= 9.3\text{x}10^{13}$ *photons / cm²·S*

where

$\alpha = \sigma N_i \eta_d \eta_f \eta$.

Specifically, for a 308 nm UV source, the power density requirement becomes

$$P_{UV}=P\frac{hc}{\lambda}=61\mu W/cm^2$$

Consequently, the excitation required to ensure sensitive detection can be met in theory using a 61 $\mu W/cm^2$ UV source. This can be provided by a filtered mercury arc, or else by one of several relatively inexpensive UV laser sources.

·*Chemiluminescent Excitation*

Chemiluminescence is the conversion of chemical energy into electromagnetic radiation. In these types of reactions, electrons are excited via a chemical reaction, and light is emitted upon return of the excited electrons to the ground state. Unlike other chemiluminescent modalities, enzyme-catalyzed 1,2-dioxetane derivatives can produce a light signal that can last from hours to days. The wavelength of emitted light is near 477 nm, and the emission can be controlled by controlling the pH.

In addition to no radioactivity exposure, sensitivity is greater for chemiluminescent methods as compared to radioactive methods. Moreover, this method is relatively simple to perform (reagents and equipment are

inexpensive). Finally this method has a low background noise level and wide dynamic range.

The SNR for the lens-less CCD approach for a 0.1 second integration time in the chemiluminescent mode of operation follows the expression obtained for the fluorescent approach and is given by (assuming negligible read noise)

$$SNR=\frac{P_e\sigma N_t\eta_d\eta_c\eta\,(A_p t)^{\frac{1}{2}}}{\sqrt{P_e\sigma N_t\eta_d\eta_c\eta+\frac{I_d}{q}}}=838$$

where P_e = enzymatic reaction turnover rate (3000 reactions/enzyme·sec); σ = enzyme to molecule coupling ratio (10 enzymes/molecular component); η_d = detection quantum efficiency (13 electrons/100 photons @ 477 nm emission); η_c = chemiluminescent quantum yield (5 photons/1000 reactions); η = CCD collection efficiency (50%). The values for the enzymatic reaction turnover rate and chemiluminescent quantum yield are typical for the dioxetane reagent.

Consequently with the above constants, the chemiluminescent approach appears feasible for detecting thousands of molecular constituents within a sample in proximity to the CCD imaging module operating at room temperature.

Lens-less Operation

Improved detection sensitivity beyond traditional CCD camera-based imaging approaches is attributable in the present invention to the coupling of the detector (CCD arrays) to the emitter (molecular sample 20). Hence by avoiding traditional optics (lenses, mirrors), the

proposed system offers a much improved collection efficiency.

The collection efficiency η is measure of an instrument's capability to capture electromagnetic radiation and is primarily a function of geometry. The geometrical configurations for lens-based instruments (epifluorescent microscope, confocal microscope, CCD camera and the like) and the lens-less CCD imaging module are illustrated in FIGS. 2A and 2B, respectively.

Collection efficiency is typically expressed as a ratio of solid angles

$$\eta = \frac{\Omega}{4\pi}$$

where

$$\Omega = \iint \frac{\cos\Psi dS}{r^2}$$

and Ψ is the angle between the surface normal vector and the position vector. For the lens configuration ($\Psi = 0$)

$$\Omega_{lens} = \int_0^{2\pi} \int_0^{\phi_0} \frac{r^2 \sin\phi d\phi d\theta}{r^2}$$

$$= 2\pi(1-\cos\phi_0)$$

where ϕ_o = half angle of the cone of rays entering the lens. Consequently, the collection efficiency of the lens-based systems is

$$\eta_{lens}=\frac{1}{2}(1-\cos\phi_0).$$

In contrast, the direct proximity lens-less CCD approach yields the largest half angle (ϕ_0 = 90°) resulting in the collection efficiency

$$\eta_{CCD}=\frac{2\pi}{4\pi}=\frac{1}{2}.$$

The difference between these configurations becomes quite apparent once numerical apertures are provided. For example, suppose a high quality lens with a numerical aperture NA = 0.3 is employed. Since

$$NA=n\sin\phi_0$$

then

$$\eta_{lens}=\frac{1}{2}\left[1-\cos\left(\sin^{-1}\left(\frac{NA}{n}\right)\right)\right]=2.3\%$$

which is substantially lower than 50% achieved by the lens-less CCD approach. Furthermore, if the numerical aperture is increased to 0.4, typical of a $100K photolithography lens for producing 1M byte memory chips, the collection efficiency increases to only 4.2%. Consequently, the integrated CCD approach provides at least a 10x collection advantage over any lens-based system, and allows the additional imaging of radioactively labeled samples.

Instrumentation Platforms

One of two preferred embodiments for housing the above described detection and imaging sensor is illustrated schematically in FIG. 3. The embodiment of FIG. 3 consists of:

a) a relatively large format imaging sensor 100 formed of a CCD module 110 comprised of a plurality of smaller imaging devices CCD1...CCDN, each device having a plurality of photosensitive pixels 102;

b) scanner apparatus 104 for relative movement of the module 110 with respect to a molecular sample 106 in a manner for efficient imaging;

c) light source 108 for exciting the molecular sample;

d) an array driver circuit 120 for driving the sensor array 110 which includes clocking, biasing, and electronic gating of the pixel electrodes in CCD1...CCDN;

e) a receiving circuit 122 for obtaining the digital image from the sensor array 110 which includes preamplification, amplification, analog to digital conversion, filtering, multiplexing, sampling and holding functions;

f) a data processor 124 for processing the imaging data including contrast enhancement and parameter estimation; and

g) display means 126 for displaying and storing the digital image.

Depending upon the sample, a variety of excitation sources 108 may be employed in the instrument. An external x-ray source used for conventional radiography can be employed for medical x-ray imaging. Also, UV excitation may be provided by a lamp or laser, mounted above or below the sample. Finally, for radioisotope and

chemiluminescent labeled samples, no excitation source is required.

The dynamic platform embodiment of FIG. 3 enables the imaging module or sample to be moved using a scanning mechanism such as a step motor mechanically coupled to platform 130. Thus by scanning, relatively large format imaging can be accomplished by a smaller sensor module as illustrated therein. A plurality of imaging devices 110 can be arranged in a module of columns to minimize discontinuity. Also, the scanning can be accomplished with intentional overlapping to provide continuous high resolution imaging across the entire large format sample area.

An additional excitation method is illustrated in FIG. 4 where a laser 200 is projected through a cylindrical lens system 202 to form a beam 204 of excitation on the sample 206 at the Brewster angle to minimize reflective losses. Reflection 208 from the concentrated line excitation is then imaged by the nearby sensor module 210 as illustrated.

Because physical contact may take place between the imaging array and the substrate sample to be analyzed, means must be used to protect the bond wires which make contact to the imaging device to connect the pixel electrodes to the driver circuits and image receiver circuits, *e.g.* 120, 122 FIG. 3. These are removed from the immediate location of the imaging array if a device with a frame store array is used; such as described in the Burke *et al.*, 1991 reference. Imaging of substrates of the same size as, or smaller than, the imaging array can be accomplished with standard wire bonding and some means to prevent the substrate from touching the device in the neighborhood of the wire bonds. However, if the size restriction of the substrate is removed and substrates which are larger than the size of the 2xN imaging array

are allowed, then special means of attaching and protecting the wire bonds must be used.

FIG. 5 shows a conventional wire bond 600 which loops above the surface of the device to bond to a contact so that contact of the wire bond with the edge 604 of the device 602, and subsequent possible shorting, may be avoided. FIG. 6 shows how the large sample substrate 700 must be flexed in order to contact the imaging array so that good resolution is assured, but also clear the epoxy encapsulated bond wire 600′ which projects typically 15 mils above the device surface. FIG. 7 shows a new method of making bonds in which the bond wires 600′′ are brought out approximately parallel to the surface of the device 602′. In order for there to be minimal chance for shorting of the bond wire to the edge of the device 602′′, a bead 701 of epoxy is first run along the edge of the device and is allowed to harden before attaching the wires. Also, the land 702 on the circuit board to which the device is bonded is positioned a distance D (the proper distance) from the edge of the device so that the wire 600′′ lies nearly flat on the device surface, protruding less than 5 mils above it, and also has a loop sufficient to relieve stresses. FIG. 8 shows the epoxy encapsulated wire 600′′′ with a substrate to be imaged 700 which is flexed to a much lesser extent than in FIG. 6.

Alternatively, as in FIG. 9, in the scanning or dynamic mode of operation, where the sample 800 is considerably larger than the sensor array of the device 701, the sample or the sensor must be moved for imaging the entire sample. Here the sensor 701 must be isolated from the sample 800 in a manner to permit relative motion, yet maintain the proximity requirements of lens-less imaging. FIG. 9 shows a method which utilizes a transparent isolation plate 22 in conjunction with the low profile bonding technique previously illustrated in FIG.

7. The distance D_i is kept minimal to enable lens-less imaging with minimal distortion.

Conversely, a backside illuminated sensor approach shown in FIG. 10 can be applied which enables the electrical connections 900 from the device package 702 to the sensor device 701 to be placed on the opposite side of the device, well away from the sample 800'. Hence a minimal distance D_b can be achieved between the sample and imaging sensor device 701 to warrant lens-less imaging as described. The backside illumination sensor approach also provides more sensitivity since the quantum efficiency is substantially improved by avoiding photo-electron transmission through the polysilicon gates of the sensor array in the device 701.

While this invention has been particularly shown and described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

1. A method of detecting and forming images representing the particle emissions in an area of a sample comprising the steps of:
   a) forming an array of particle detectors, said array having a particle detector area of size commensurate with the area of the sample to be imaged;
   b) converting, in said detectors, said particle emissions emanating directly from said sample into photoelectrons;
   c) collecting said photoelectrons in said detectors; and
   d) forming an image of said collected electrons.

2. The method of Claim 1 in which said array is disposed in close proximity to said sample.

3. The method of Claim 2 in which no intervening lens structure is provided between the sample and the array.

4. The method of Claim 1 in which the array is formed of charge coupled devices.

5. The method of Claim 1 in which the array of particle detectors comprises flat panels within which an array of charge-coupled devices are formed and which panels are disposed in close proximity to said sample.

6. The method of Claim 1 in which the panels contact the sample.

7. The method of Claim 1 in which the samples are labelled with substances from the group comprising radioisotope, fluorescent or chemiluminescent molecules.

8. The method of Claim 1 in which the sample is moved with respect to the array.

9. The method of Claim 1 wherein the particle detectors are taken from the group comprising CCD's, charge injection devices, amorphous silicon sensors, and photodiodes.

10. A method of detecting and forming images representing the particle emissions in an area of a sample comprising the steps of:

a) contacting the sample with an array of particle detector devices;

b) converting, in said detector devices, said particle emissions emanating directly from said sample into photoelectrons;

c) collecting said photoelectrons in said device; and

d) forming an image of said particles from said collected photoelectrons.

11. The method of Claim 10 in which said array is formed of devices from the group comprising CCD's, CID's, amorphous silicon sensors, and photodiodes.

12. The method of Claim 10 in which the sample and the array are moved with respect to one another.

13. The method of Claim 10 in which the sample is labelled with material from the group comprising radioisotope, fluorescent or chemiluminescent molecules.

14. Apparatus for forming an image representing particle emissions in an area of a sample comprising a particle detector arranged proximal with the area of the sample to be imaged; each detector converting particle emissions, incident directly, without passing through a lens, from adjacent sample portions, into photoelectrons and collecting said photoelectrons; and a display for forming an image of said detected particles from said collected photoelectrons.

15. The apparatus of Claim 14 wherein the image is a spatial image.

16. The apparatus of Claim 14 wherein the image is a temporal image.

17. The apparatus of Claim 14 wherein the particle detector is a solid state charge-coupled device.

18. The apparatus of Claim 14 including a scanner to move the detector array or sample relative to one another.

19. Apparatus for forming an image representing particle emissions in an area of a sample comprising an excitation source for exciting the sample into emitting particles, a particle detector arranged within an area of size commensurate with the area of the sample to be imaged; each detector converting particle emissions, incident directly from adjacent

sample portions, into photoelectrons and collecting said photoelectrons; and a display for forming an image of said collected photoelectrons.

20. The apparatus of Claim 19 wherein the sample is in contact with the detector.

21. The apparatus of Claim 19, wherein the excitation source is a UV light source, the detector is a CCD with a polysilicon gates and an optical filter.

22. The apparatus of Claim 19 wherein the particle detector is a solid state charge-coupled device.

23. The apparatus of Claim 19 including a scanner to move the detector array or the sample relative to one another.

24. Apparatus for detection and quantification of particles emitted from a sample to produce a digital representation of the detected particles comprising an array of detectors for converting emissions from said sample to electrical signals over a wavelength range from about 1 to 10,000Å, and a dynamic range of at least 5 orders of magnitude and wherein said array is disposed proximal to said sample in the path of the emissions without any intervening lenses.

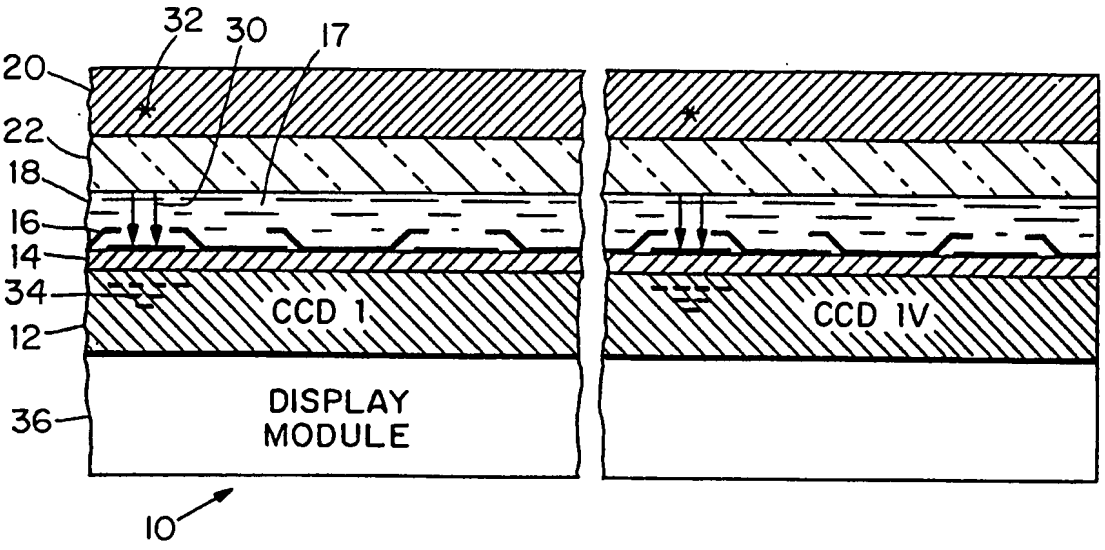

FIG. 1A

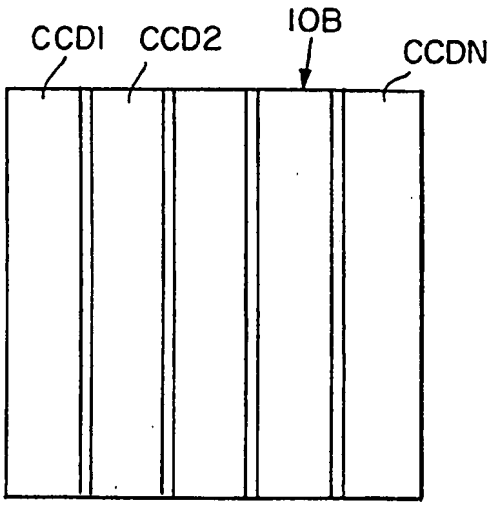

FIG. 1B

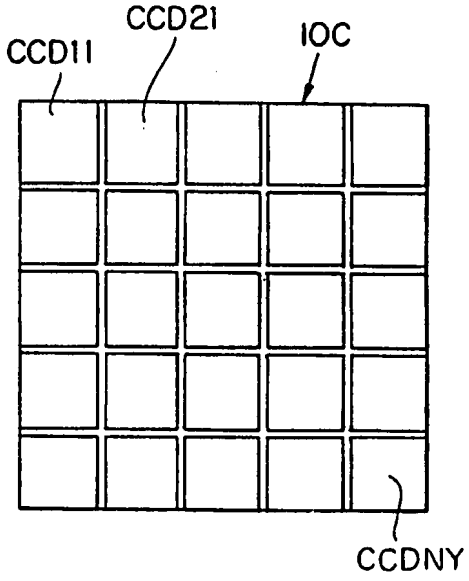

FIG. 1C

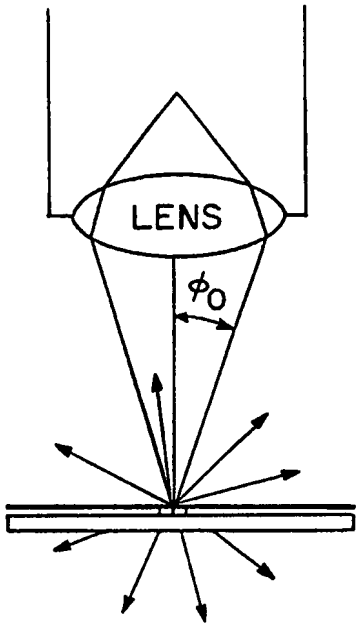

FIG. 2A
PRIOR ART

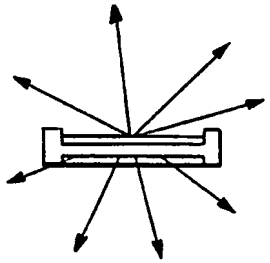

FIG. 2B

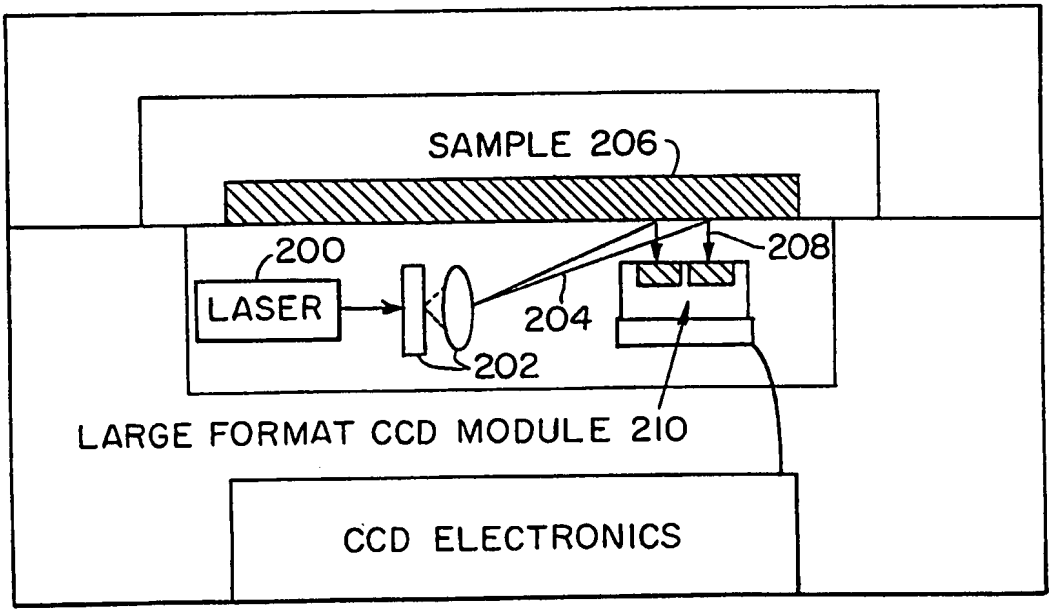

FIG. 4

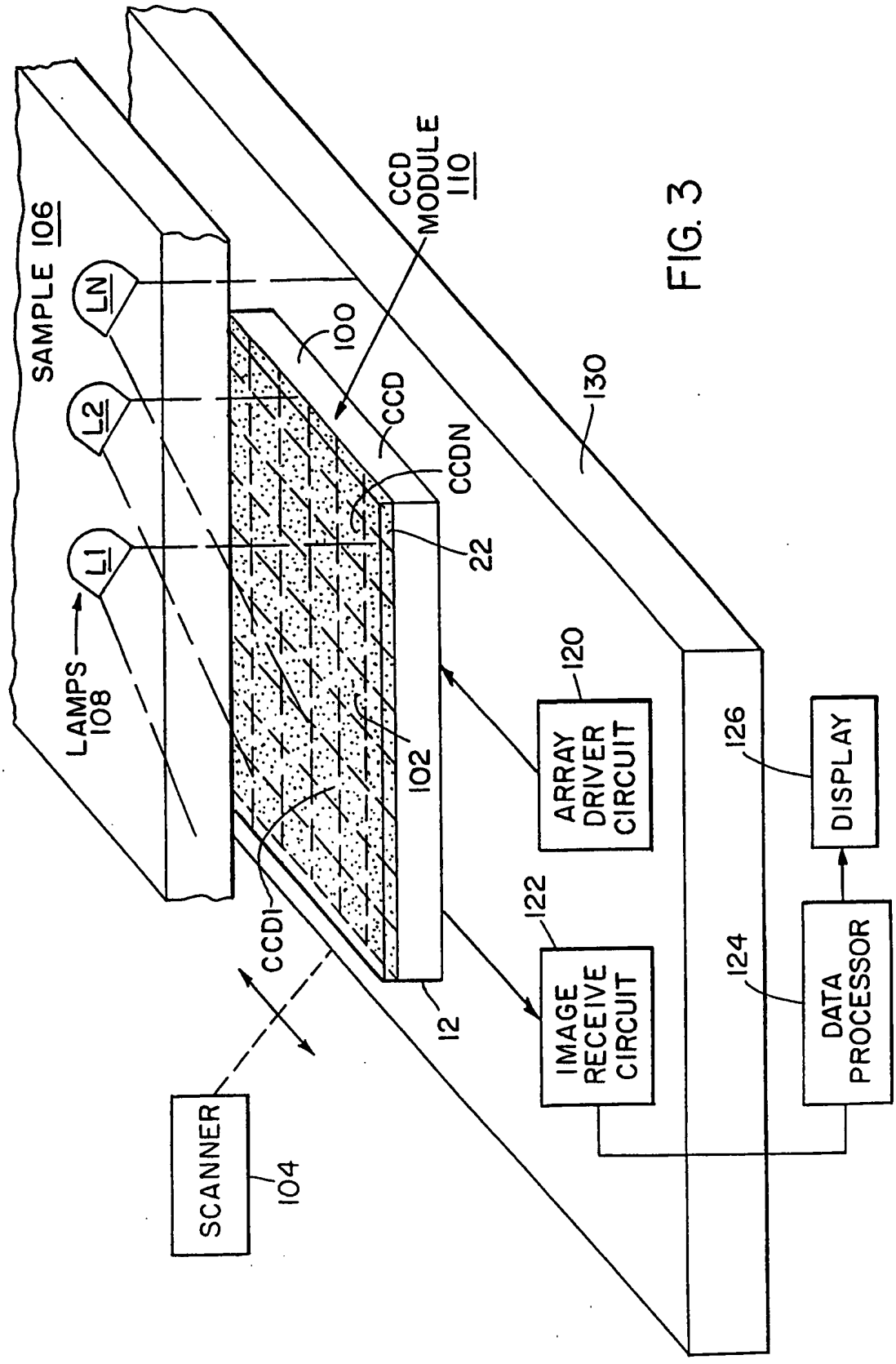
SAMPLE 106
LN
L2
L1
LAMPS 108
CCD MODULE 110
100
CCD
CCDN
22
130
102
ARRAY DRIVER CIRCUIT
120
126
DISPLAY
122
IMAGE RECEIVE CIRCUIT
124
DATA PROCESSOR
CCD1
12
SCANNER
104

FIG. 3

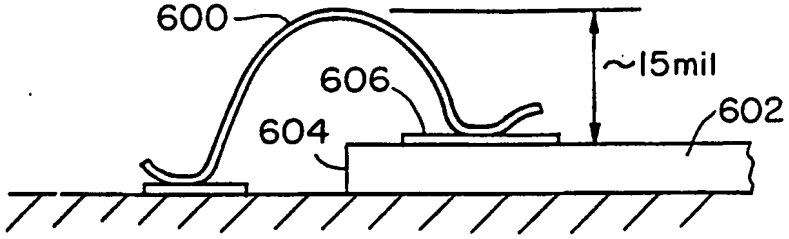

FIG. 5
PRIOR ART

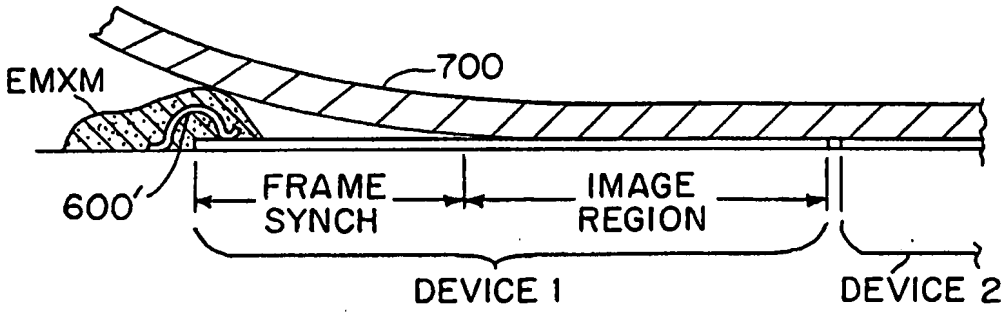

FIG. 6

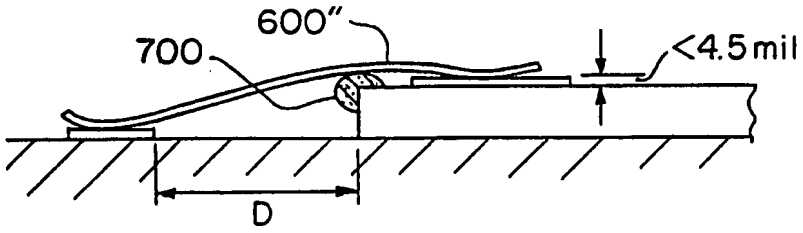

FIG. 7

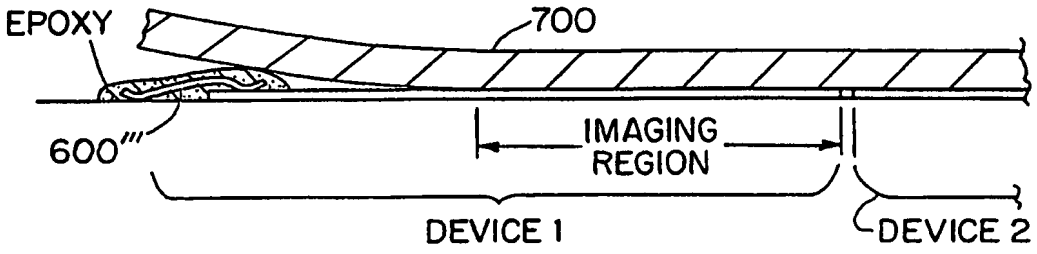

FIG. 8

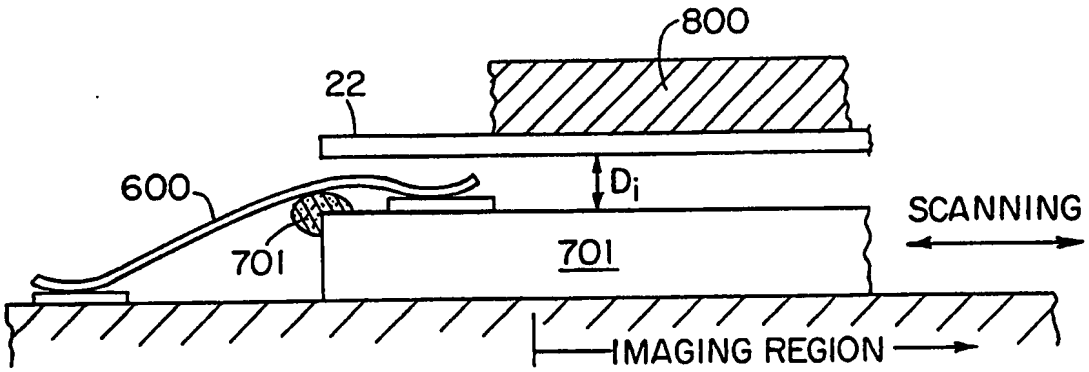
800
22
600
D_i
SCANNING
701
701
IMAGING REGION

FIG. 9

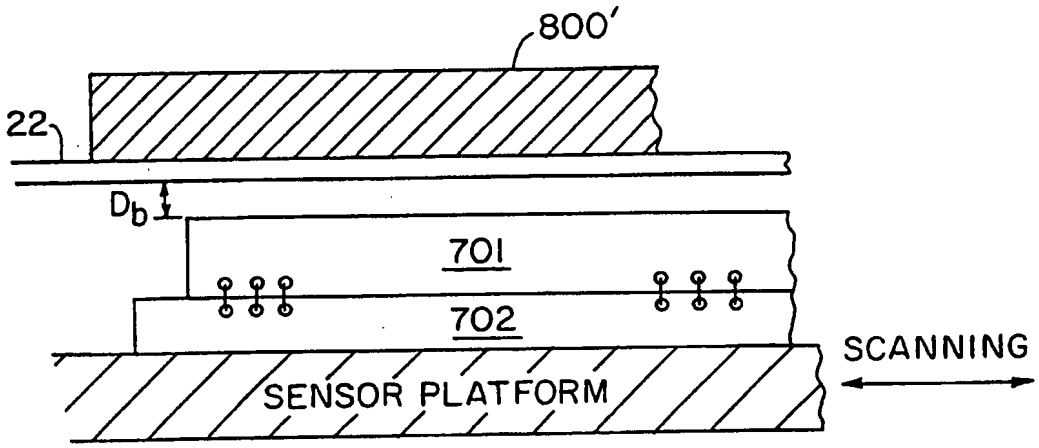
800'
22
D_b
701
702
SENSOR PLATFORM
SCANNING

FIG. 10

INTERNATIONAL SEARCH REPORT

Interna l Application No PCT/US 95/01725

A. CLASSIFICATION OF SUBJECT MATTER
G 01 T 1/29,G 01 T 1/164

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A 61 B,G 01 T,G 03 G,H 04 N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELECTROPHORESIS, vol. 9, issued 1988, JACKSON P. et al. "Rapid imaging, using a cooled CCD, of fluorescent two-dimensional polyacrylamide gels produced by labelling proteins in the first-dimensional isoelectric focusing gel with the fluorophore 2-methoxy-2,4-diphenyl-3(2H)furanone" pages 330-339, the whole document, especially 2.9 (cited in the application).	1,4,8, 9,19, 23
A	--	20,21, 22

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 1995	26. 07. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	WERNER e.h.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/01725

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANALYTICAL CHEMISTRY, vol. 63, no. 1, issued January 1991, SWEEDLER J.V. et al. "Fluorescence Detection in Capillary Zone Electrophoresis Using a CCD with Time-Delayed Integration" pages 496-502, the whole document, especially fig. 1-4 (cited in the application). --	1,4,7, 8,9, 19
X	US, A, 4 882 494 (ROGERS et al.) 21 November 1989 (21.11.89), the whole document, especially fig. 1,2. --	1-3,8, 14-16, 18
X	ANALYTICAL CHEMISTY, vol. 63, no. 7, issued April 1991, CHAN K.C. et al. "On-Line Detection of DNA in Gel Electrophoresis by Ultraviolet Absorption Utilizing a CCD Imaging System" pages 746-750, the whole document, especially page 747, column 2, lines 35-62.	19
A	--	20-23
X	EP, A, 0 583 118 (SUMMIT WORLD TRADE CORPORATION) 16 February 1994 (16.02.94), the whole document, especially claims 1-11; fig. 1-5. --	1-3
X	EP, A, 0 421 869 (COMMISSARIAT A L'ENERGIE ATOMIQUE) 10 April 1991 (10.04.91), the whole document, especially abstract; column 2, lines 36,37.	1
A	----	2,24

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 95/01725 SAE 105734

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
US A 4882494	21-11-89	AU A1 17939/88	22-09-89
		EP A1 441772	21-08-91
		IL A0 88922	15-08-89
		WO A1 8908268	08-09-89
EP A2 583118	16-02-94	keine - none - rien	
EP A1 421869	10-04-91	CA AA 2026808	05-04-91
		FR A1 2652655	05-04-91
		JP A2 3137589	12-06-91